Remarks

Claims 1-35 are pending in this application. Claims 1-17 and 35 are withdrawn from consideration as being drawn to non-elected subject matter. Claims 1-35 have been rejected. Claims 1-2, 5, 7-16, 18-19, 22, 24-33 have been amended. Claims 17, and 34-35 have been cancelled. The amendments are supported by the specification and add no new matter.

Claims rejections under § 112, second paragraph

Claims 26 and 28 have been rejected as being indefinite because of the recitation of the phrase "glycine synthetase leader." Claims 26 and 28 have been amended to replace "glycine synthetase leader" with "*glyQS* leader." The amendment overcomes the rejection.

Claim rejections under 35 U.S.C. § 103

Claims 18-34 have been rejected as being obvious and unpatentable over van de Guchte et al. (2001) in view of Landick et al. (1996), Kirschbaum et al. (USPN 6,174,722), Edwards et al (USPN 5,578,444) and Schimmel et al (1998).

Independent claims 18 and 30, as amended, recite a purified ***in vitro*** assay system comprising a ***glyQS leader*** sequence. Applicant submits that independent claims 18 and 30, as amended, are ***not*** obviousness. There is simply no teaching or guidance in the general knowledge of the relevant art at the time the application was filed regarding how to make an *in vitro* assay directed specifically at inhibition of T-box regulated genes. More particularly, there is no teaching or guidance anywhere in the cited art as to how to make such an *in vitro* assay system and there is absolutely no mention or even suggestion of such a system using the *glyQS* leader.

Essential to the success of the *in vitro* assay system of the instant claims was Applicant's discovery, no where mentioned or even suggested in van de Guchte or any of the other cited art, that *glyQS* would be suitable for use in an *in vitro* assay directed specifically at inhibition of T-box regulated genes. Applicant first recognized and reported that *glyQS* is a natural deletion variant that lacks the Stem II and Stem IIA/B pseudo-knot elements -- elements that are highly conserved in other members of the T box family of genes (application at page 16, lines 3-4). And Applicant first discovered and reported that the deletion variants are uniquely suitable for

successful demonstration of tRNA-directed antitermination *in vitro*. There is no recognition, much less any mention, in van de Guchte or any of the other cited art that *glyQS* represents a special class of T-box leaders that is uniquely suitable for *in vitro* analysis of tRNA-directed antitermination. Furthermore, there is no mention or recognition in van de Guchte or any of the other cited art that $tRNA^{Gly}$, which is the tRNA species responsible for antitermination of the *glyQS* gene, is unmodified in its anticodon domain, a feature that allows utilization of tRNA generated by *in vitro* transcription using T7 RNA polymerase in the assay system for *glyQS* antitermination. Thus, in further lacking the appreciation of the role of $tRNA^{Gly}$, van de Guchte nor any of the other cited art provides any guidance whatsoever as to which T-box regulated gene leader would work in an *in vitro* assay according to the instant claims.

The Office has stated that van de Guchte "taught that *B. subtilis glyQS* and its corresponding regulatory $tRNA^{Gly}$ fell into this family of T-box regulated genes," citing Fig. 6. Applicant respectfully counters that van de Guchte does not provide any teaching regarding the use of *glyQS*. Indeed, beyond what is shown in Fig. 6, the only mention of *glyQS* in van de Guchte constitutes speculation, based on the conserved structural features of a variety of other leader transcripts, that *glyQS* ***may be*** a member of the T-box family. This speculation was not verified or pursued experimentally by van de Guchte. Nowhere in van de Guchte is there a mention of the unique features of *glyQS,* and most importantly, nowhere in van de Guchte is there any mention or suggestion that *glyQS* would be a suitable component for an *in vitro* assay system. Applicant submits that the mere knowledge of a number of genes that ***may*** belong to a particular family, such as the family of T-box regulated genes, in no way provides any teaching or prediction as to ***which*** gene or genes actually belong to that family or how they would function under specific assay conditions. Moreover, in the instant case, the mere listing in van de Guchte of a number of potential T-box regulated genes, without any recognition of structural features that influence functionality in a particular assay context, such as in an *in vitro* assay, in no way constitutes a teaching or prediction that *glyQS* is indeed uniquely suitable for an *in vitro* assay system.

The other cited references and general knowledge in the field does not provide the teaching or suggestion missing from van de Guchte. Prior to Applicant's work, it was not known what compliment of assay components or other factors were required to mediate the tRNA-

leader interaction. It was not even known whether the tRNA acted alone or in combination with trans-acting factors required to mediate the leader RNA-tRNA interaction. (Application, p. 10, last ¶ to p. 11, 1st ¶)). In fact, based on what was known in the art at the time of the instant application, a person of ordinary skill in the art would have expected that the leader RNA-tRNA interaction *in vivo* may involve additional determinants (App., p. 10, 3rd ¶ and cited references). The fact that prior to the present application no other investigator had successfully implemented an *in vitro* T box antitermination assay using completely purified components is evidence that making such an assay system work required more than mere "modification" of what was known in the art. (see, e.g., provisional application at p. 7, 2nd ¶, enumerating studies where others have tried and failed to make the T-box mechanism work *in vitro*). It was Applicant who, for the first time, disclosed "***a factor- and ribosome-independent antitermination*** of the *B. subtilis glyQS* leader ... in a ***purified in vitro*** transcription system." (App., p. 11, 1st ¶).

As for the presence of Mg^{2+} in the assay mixtures, the claims as amended recite that the Mg^{2+} in the *in vitro* assay system is present in a concentration of at least 30 mM -- a concentration that is a great deal higher than that present *in vivo*. Therefore, contrary to the Office's assertion, the Mg^{2+} in the assay system would ***not*** have inherently been present in the cells of *L. lactis* used in the van de Guchte study.

In short, not only does van de Guchte fail to teach every element of the claimed invention, but it fails to provide any teaching, suggestion, or guidance, as to ***how*** to convert the *in vivo* T-box mechanism into a working and effective purified *in vitro* assay for identification of potentially inhibitory substances.

Applicant submits that Landick, and the additional cited art, does not provide what van de Guchte lacks. The Office, in rejecting the instant claims, has attempted to explain how Landick addresses the difference between the *in vivo* assay of van de Guchte and the claimed assay mixtures by stating:

> Landick taught advantages of using dinucleotides and halted-complexes .... Since these advantages would only have been attainable by modifying the teachings of van de Guchte to make in vitro rather than in vivo assay mixtures, it would have been prima facie obvious to one of ordinary skill in the art to do so." (O.A., p. 9 last p to p. 10, 1st ¶).

Applicant submits that this assertion by the Office is a clear hind-sight reconstruction. A person of ordinary skill in the art, ***at the time the invention was made***, and without the benefit of any guidance regarding which leader would work in an *in vitro* assay, would not have known how to "modify" the *in vivo* system of van de Guchte to arrive at an *in vitro* assay, with or without Landick, because Landick does not provide any guidance as to which particular leader RNA/tRNA combination would be necessary and sufficient for a successful *in vitro* assay of a T-box regulated gene.

In addition, Landick with van de Guchte does not render obvious claims 29-34, which recite the halted-complex assay, because Applicants used the halted-complex assay ***not for the advantages cited by Landick*** (i.e. "(i) greater synchrony during the elongation phase… and (ii) the ability to end-label the RNA transcript." O.A p. 10), but because the halted-complex assay allowed the use of conditions (e.g. high Mg^{2+}) during the elongation phase that are inhibitory during the initiation phase. (App. p. 15, Example 3, explaining that no tRNA-dependent read-through was observed at low Mg^{2+} concentration). The use of high Mg^{2+} during the elongation phase is a requirement for *glyQS* antitermination that was not predictable from the cited art. Furthermore, Landick describes the use of the halted complex assay for measurement of pausing and not for transcription termination and antitermination, as recited in the instant claims. In sum, the Office fails to explain why the difference between the *in vivo* system of van De Guchte and the claimed *in vitro* assay would have been obvious in view of Landick given the very different information sought through the Landick assay as compared with the assays of the instant claims.

Similarly, neither Kirschbaum nor Edwards provides the teaching or suggestion missing from van de Guchte. The Office's has asserted that:

> it would clearly have been *prima facie* obvious to one of ordinary skill in the art ... to *modify* the teachings of van de Guchte to make assay mixtures with and without a *potential inhibitor substance* (as taught by Kirschbaum) for the benefit of screening for molecules that could *interfere with transcription*, and thus identify useful antibiotics (as taught by Edwards). (O.A., p. 9, 1st ¶)

The Office's reliance on Kirschbaum and Edwards is misplaced for two reasons: first, because, as compared to the assays of the instant claims, these references are assessing different mechanistic aspects of transcription in general; and second, because these two references address inhibition of transcription ***initiation***, not inhibition of transcription ***antitermination*** as claimed.

(See Kirschbaum at col. 6, lines 41-58; Edwards at Col. 65, lines 48-52). These are two completely different mechanisms that occur under completely different circumstances and with different components and reagents. In view of these known differences, Applicant submits that the mere fact that "potential inhibitor substances" can be identified in an *in vitro* transcription initiation assay ***does not*** make it predictable that potential inhibitors of antitermination could be similarly identified in an *in vitro* assay. This is particularly so since none of the cited art recognized the importance of *glyQS* or the feasibility of an *in vitro* antitermination assay.

Finally, the Office has attempted to explain the difference between van de Guchte (which did not teach the use of *B. subtilis glyQS* and $tRNA^{Gly}$ in an assay mixture) and the claims by citing Schimmel, which according to the Office, stands for the position that "aminoacyl tRNA synthetases are being pursued as targets for new drugs. .... The key to their usefulness lies in being able to find drugs that inhibit a pathogen synthetase but not its human cell counterpart." (O.A. p. 9, 2nd ¶).

Applicant submits that Schimmel is irrelevant, and certainly does not provide any teaching that is lacking in the other references. Schimmel addresses drugs that interfere with aminoacyl-tRNA synthetase enzyme activity through interaction or binding with the enzyme itself (Schimmel at p. 1604, col. 2, "Pathogen specific inhibitors based on reaction intermediates;" p. 1606, 1st ¶, describing targeting drugs to the catalytic sites of enzymes; or p. 1606, 2nd ¶, describing compounds that block protein-RNA domain-domain interactions"). Schimmel is completely silent on targeting the T-box mechanism as a potential target site. Furthermore, merely recognizing the desirablity to have a thearpeutic agent and an assay system for identifying such an agent does not substitute for specific guidance on how to configure such an assay system. And with respect to the instant claims, the recognition that pathogen specific inhibitors may target interactions with RNA ***does not*** substitute for teaching which RNA interactions and which specific reaction components are needed to provide an *in vitro* T-box antitermination assay. At most the cited art could be argued to suggest the T-box mechanism as an attractive potential target. But ***implementation*** of the concept of targeting the T-box mechanism by providing a functional *in vitro* assay of the T-box mechanism is simply not taught by the cited art, and there is no hint or suggestion as to what specific components would be needed for such an assay system.

In sum, van de Guchte, neither alone nor in any combination with Landick, Kirschbaum, Edwards and Schimmel, provides any teaching, suggestion, or guidance as to which particular synthetase under which particular conditions can be utilized in an *in vitro* assay targeting the T-box mechanism in bacteria. Thus, the Office has not established *prima facie* obviousness because it has misconstrued the scope and content of the art, the differences between the *in vivo* conditions of the art and the claimed *in vitro* assays, and has failed to explain how a person skilled in the art would have found it obvious to arrive at the claimed invention from either the art teachings, the general knowledge in the art, or common sense.

Claims 19-29 and 31-33 depend from claims 18 or 30 and so are also nonobvious.

In view of the amendments and remarks, applicants respectfully submit that the claims are now in condition for allowance. Prompt notice of such allowance and rejoinder of claims 1-16 are respectfully requested. If the Examiner believes that other language is need to overcome the rejections, or any other questions regarding the amendments or remarks, she is encouraged to contact Diane Dobrea at (614) 621-7788.

Respectfully submitted,

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